

# Laboratory Evaluation of a Manure Additive for Mitigating Gas and Odor Releases from Layer Hen Manure

Ji-Qin Ni<sup>1\*</sup>, Albert J. Heber<sup>1</sup>, Teng T. Lim<sup>1,2</sup>, Sam M. Hanni<sup>1</sup>, Claude A. Diehl<sup>1</sup>

<sup>1</sup> *Department of Agricultural and Biological Engineering, Purdue University, West Lafayette, Indiana 47907, USA*

<sup>2</sup> *Current address: Division of Food Systems and Bioengineering, University of Missouri, Columbia, Missouri 65211, USA*

## Abstract

Manure additives are widely used to mitigate gas and odor emissions from manure or improve manure properties. However, the reported effectiveness of some manure additive products has been mixed and most of the studies on poultry manure have been on chemical additives. A laboratory study was conducted to evaluate an enzyme-based commercial manure additive for its potential reductions of ammonia (NH<sub>3</sub>), carbon dioxide (CO<sub>2</sub>), hydrogen sulfide (H<sub>2</sub>S), and odor releases from layer hen manure. Eight 122-cm tall and 38-cm diameter reactors, four treated with the additive and four control, were studied for 38 days with manure from commercial layer hen houses. The reactors were initially filled with 66-cm height manure followed by weekly additions of 5 cm each. Ventilation air was supplied to the reactor headspace to simulate winter ventilation rates in layer hen houses. Concentrations of NH<sub>3</sub>, CO<sub>2</sub>, and H<sub>2</sub>S in the reactor exhaust air were measured with gas analyzers for 10 min, six times daily. Odor intensity was assessed by a trained odor panel. Open-headspace tests were also conducted to corroborate the observations in the reactor study. Study results showed that the average 4-reactor group mean release rates  $\pm$  standard deviations of NH<sub>3</sub> were  $17.3 \pm 15.1$  and  $19.9 \pm 13.4 \mu\text{g s}^{-1}$  from the control and treated groups, respectively. Those of CO<sub>2</sub> were  $1086 \pm 157 \mu\text{g s}^{-1}$  from the control and  $1146 \pm 237 \mu\text{g s}^{-1}$  from the treated groups. Release of H<sub>2</sub>S from the reactors could not be detected. The odor intensities were  $3.5 \pm 0.3$  and  $3.4 \pm 0.3$  before and after the additive spray, respectively. Application of the additive onto the manure did not demonstrate effect on the releases of NH<sub>3</sub> ( $P > 0.41$ ), CO<sub>2</sub> ( $P > 0.21$ ), and odor ( $P > 0.71$ ) from the manure.

**Keywords:** Agricultural wastes; Emission mitigation; Manure treatment; Poultry manure; Waste management.

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\* Corresponding author: Tel.: +1-765-496-1733; Fax: +1-765-496-1115  
E-mail address: jiqin@purdue.edu (J.-Q. Ni)

## 32 INTRODUCTION

33 Modern livestock and poultry production facilities are becoming larger and more  
34 concentrated, resulting in greater public scrutiny and stricter government regulations. Emissions  
35 of certain aerial pollutants, especially ammonia (NH<sub>3</sub>), and odors from poultry facilities have  
36 been an ecological and environmental concern. Average NH<sub>3</sub> emission rates from high-rise layer  
37 hen houses range from 0.60 to 1.28 g d<sup>-1</sup> hen<sup>-1</sup> at various locations in the U.S. (Heber et al., 2005;  
38 Liang et al., 2005; Lin et al., 2012; Wang-Li et al., 2013). Odor emissions from layer hens were  
39 59.3 OU<sub>E</sub> s<sup>-1</sup> AU<sup>-1</sup> (OU<sub>E</sub> = European odor unit; AU = Animal unit or 500 kg live mass) from two  
40 high-rise houses in Indiana (Heber et al., 2005) and 46.7 OU<sub>E</sub> s<sup>-1</sup> AU<sup>-1</sup> from two manure-belt  
41 houses in Ohio (Zhao et al., 2015).

42 Manure additives are widely used in livestock and poultry farms. Commercial manure  
43 additives are claimed to reduce NH<sub>3</sub> and hydrogen sulfide (H<sub>2</sub>S) emissions, combat odors and/or  
44 odor production, break down solids, and increase the availability of manure nutrients. In the U.S.,  
45 manure additives were introduced as early as in the 1910s. For example, acid phosphate was  
46 recommended to preserve nutrients in poultry manure by the Maine Agricultural Experiment  
47 Station (Pearl, 1913). There are currently about 50 commercial proprietary additive products  
48 available in the U.S., in addition to general chemicals such as aluminum sulfate and aluminum  
49 chloride. These additives come in a variety of forms including chemicals, bacteria, enzymes, and  
50 other biological products. Investigations have been conducted on different additives and reports  
51 published with mixed results. The largest laboratory study of manure additives so far was an  
52 evaluation of 35 commercial manure additives for controlling odor, NH<sub>3</sub>, and H<sub>2</sub>S releases from  
53 swine manure in three consecutive 42-d trials. Results showed that, at 95% certainty, only 23% of  
54 the products reduced NH<sub>3</sub>, 20% of the products reduced H<sub>2</sub>S, and no products reduced odor  
55 (Tengman et al., 2001). Another study of three additives in swine manure showed only very

56 limited odor and solids reductions (Stinson et al., 2000). Based on different research results,  
57 Lorimor et al. (2002) concluded that manure additives are generally not reliable for emission  
58 controls. However, a couple of more recent studies demonstrated that some additives showed  
59 improvement in air quality (Shah et al., 2007) and odor reduction (Brandt et al., 2016).

60 Although testing of additives to reduce  $\text{NH}_3$  and odor emission from chicken manure has  
61 been conducted since the early 1920s (e.g., Collison and Conn, 1922), relatively fewer studies on  
62 poultry manure were available in the literature (e.g., King et al., 2006; Tasistro et al., 2007)  
63 compared with those on swine manure (e.g., Yu et al., 1991; Alkanani et al., 1992; Li et al., 1998;  
64 Heber et al., 2000; Zhu et al., 2006). In addition, most of the studies on poultry manure evaluated  
65 chemical acidifiers to reduce ammonia emissions (e.g., Lim et al., 2008; Bejan et al., 2013).

66 A commercial manure additive Eco-Cure™ (Eco-Cure, Inc., Corte Madera, CA) has been  
67 in the U.S. market for more than a decade. It was advertised as an enzyme-based product for  
68 livestock and poultry producers to rapidly reduce  $\text{NH}_3$  and odor releases upon contact with  
69 animal manure. In search for an air pollution abatement technology for layer hen houses to satisfy  
70 the abatement requirements of a Consent Decree with U.S. EPA (United States Environmental  
71 Protection Agency) (DOJ, 2004), an egg producer sponsored a laboratory study of this additive to  
72 determine its effectiveness on air pollution reduction. The producer desired a successful test to  
73 minimize the cost of reducing  $\text{NH}_3$  emissions from their facilities; but the U.S. EPA demanded  
74 that preliminary data be collected before conducting the required field tests that could ultimately  
75 satisfy legal requirements. The U.S. EPA supervised the laboratory study to assure quality  
76 assurance and quality control. The objective of this paper is to report the effectiveness of Eco-  
77 Cure on reducing  $\text{NH}_3$  and odor releases from stored layer hen manure under laboratory test  
78 conditions.

## 79 MATERIALS AND METHODS

### 80 Study design and laboratory facility

81 A 38-d study was designed to evaluate the additive with layer hen manure (Table 1). The  
82 manure was placed into eight vertical rigid PVC reactors, including four controls denoted as R-a  
83 to R-d and four treated denoted as R-e to R-h.

84 Table 1: Laboratory study schedule.

Study day	Manure operation/additive testing	Manure height*, cm	Analyzer calibration	Additive application
-1	Collection from layer hen house		√	
0	Filling and sampling**	66.0	√	√
7	Addition	71.1	√	√
14	Addition and odor evaluation	76.2	√	√
21	Addition and open-headspace test	81.3	√	√
28	Addition and open-headspace test	86.4	√	√
31		86.4	√	
35		86.4	√	√
37		86.4	√	
38	Sampling and emptying**		√	

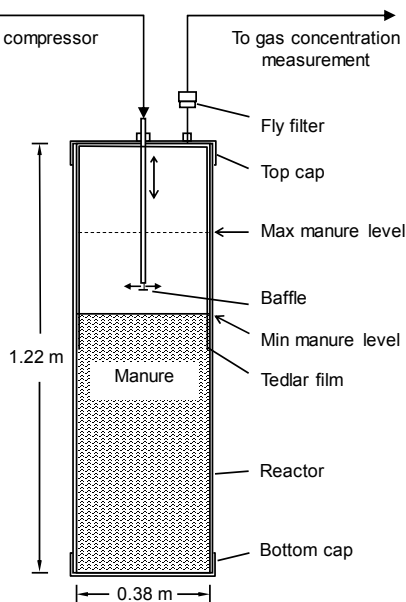
85 Note: \*Manure height was the distance from the reactor bottom to the manure surface;  
86 \*\* Samples were for manure analysis.

87 The reactors were 1.22 m tall and 0.38 m inside diameter with slip caps on each end. Each  
88 reactor was lined with 0.05-mm thick Tedlar® film on the top 64 cm of the inside walls and the  
89 “ceiling” of the reactor (inside the top slip cap) to create a chemically inert headspace (Figure 1).

90 The air inlet opening of the reactors was adjustable and telescoping to allow the inlet to  
91 always be located 15 cm above the manure surface. The air inlet included a baffle to direct the air  
92 radially in all horizontal directions so that the incoming air did not blow directly onto the manure  
93 surface. The eight reactors were placed in a 4.5 m x 2.7 m insulated and environmentally  
94 controlled walk-in test chamber, which was maintained at about 20°C.

95 An air compressor provided ventilation air to the reactors continuously except during  
96 manure addition and additive application (Figure 2). Pressurized air was filtered; and the pressure  
97 was reduced and stabilized after going through two pressure regulators that were connected in

98 series. An air supply manifold ( $M_a$ , Figure 2) distributed air equally to each reactor at  $7.0 \text{ L min}^{-1}$   
99 using 0.84-mm diameter stainless steel precision orifices. An airflow rate of  $7.0 \text{ L min}^{-1}$  was  
100 selected to simulate similar air exchange as in layer hen houses during cold weather.



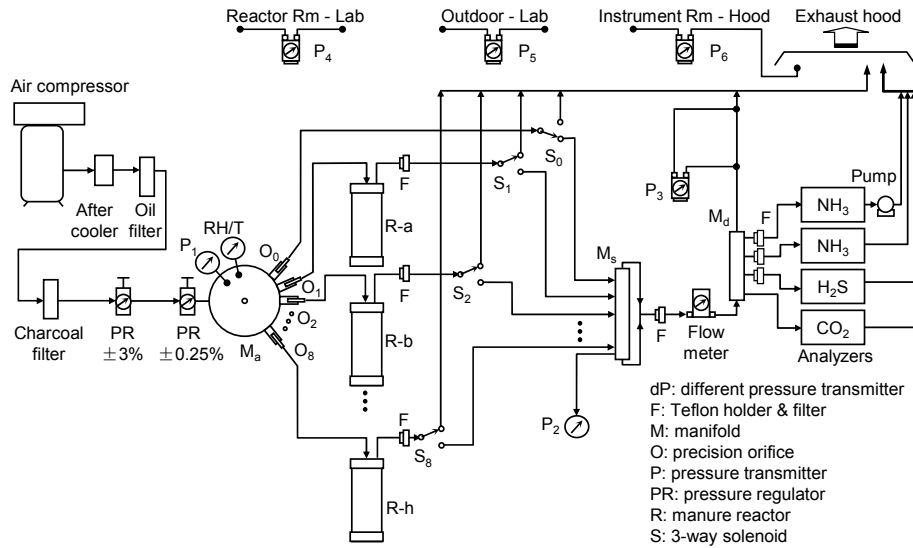
101

102 Figure 1: The reactor. The “top cap” was removed during manure addition, additive spray, and  
103 reactor emptying. The position of the air supply pipe was adjustable (indicated with the vertical  
104 double-ended arrow).

105 A Teflon® filter holder with a foam dust filter was installed in each reactor to remove  
106 manure flies from exhaust air. An air sampling setup, constructed for automatic sequential  
107 sampling, allowed exhaust air from a selected reactor or the fresh air from  $M_a$  to pass through a  
108 solenoid to a ported Teflon sampling manifold ( $M_s$ , Figure 2). The exhaust air from each reactor  
109 flowed under pressure through a 6-m long Teflon tube to a computer-controlled array of nine 3-  
110 way Teflon-lined solenoids ( $S_0$  to  $S_8$ , Figure 2) in the instrumentation room, which was  
111 immediately adjacent to the test chamber.

112 The common port of the solenoid was connected to the reactor exhaust air. The normally  
113 closed port was connected to the air exhaust located under an exhaust hood. The reactor exhaust  
114 air was discharged to the outdoors when the airflow rate and gas concentrations of that reactor  
115 were not being measured. The normally-open port of the solenoid was connected to the sampling

116 manifold,  $M_s$ . The air flowing directly from the air supply manifold, controlled by the solenoid  $S_0$ ,  
 117 was also sampled to provide a blank air check during each sampling cycle.



118  
 119 Figure 2: Laboratory setup airflow diagram. Three of the gas analyzers had internal filters and/or  
 120 pumps.

121 **Manure and manure addition**

122 Layer hen manure was collected from a commercial high-rise layer hen house in Ohio state  
 123 by the laboratory study sponsor. The house (House 1) was 201 m (L) by 21 m (W) and had a  
 124 capacity of 170,000 layer hens. Hen manure dropped in the manure pit onto eight 185-m long  
 125 manure windrows. The manure for the test was taken from the surface to the core of two manure  
 126 windrows that had accumulated for about six months. The manure was collected in the early  
 127 morning of day -1 before the study began, sealed into plastic bags and placed into cardboard  
 128 boxes, and delivered to the testing lab on the same day.

129 The initial reactor filling was conducted on day zero (0). The boxes of manure, each  
 130 weighing between 12.7 and 20.0 kg, were randomly selected for filling into each reactor until the  
 131 height of manure reached 66 cm. The average quantity of manure added was 45.1 kg per reactor  
 132 and the average manure density was 596 g L<sup>-1</sup>.

133 Bags containing 3.4 kg manure each were also prepared on day 0 for subsequent weekly

134 additions and the bagged manure was kept frozen. To simulate field conditions, 5 cm of thawed  
135 manure (one bag of 3.4 kg) was added to each reactor every week during the first three weeks.  
136 Due to insufficient quantity of source manure collected on day -1, more manure was collected on  
137 day 27 from another house (House 2) at the same farm for the fourth addition on day 28. The  
138 manure collected on day 27 was not kept frozen before being added into the reactors.

139 Three samples were taken from the source manure on day 0 before initial filling. Samples  
140 of the day 28 source manure were not available due to a test error. To make up the lost data, one  
141 manure sample was taken from the top manure (manure added on day 28) in each reactor on day  
142 38 immediately after the reactor top lid was opened. The manure in each reactor was then  
143 emptied into a tub and mixed. Three manure samples were taken from the mixture for each  
144 reactor. The manure samples were analyzed for pH, and concentrations of moisture, total nitrogen,  
145 and ammonium in the Animal Sciences Waste Management Laboratory at Purdue University.

#### 146 **Additive preparation and application**

147 The manure additive was supplied by the laboratory study sponsor. Eco-Cure Inc. provided  
148 instructions about the additive preparation and application dosage directly to the research team of  
149 this study before and during the study. To prepare the additive that was originally in solid form,  
150 15 g of additive were submerged into 2 L of 35°C water, which contained less than 0.5 ppm  
151 chlorine and had a pH of 7.02, for at least five hours to obtain a solution that looked similar to  
152 brown tea.

153 The additive solution applied to each treated reactor was 35 mL for the initial application  
154 and 15 mL for each of the weekly applications. Nothing was applied to the control reactors. The  
155 solution was sprayed onto the manure surface inside the treated reactors as uniformly as possible  
156 using a hand pump spray, which consistently sprayed 2.5 mL of liquid every squeeze of the pump.  
157 The weekly application started after the manure addition operation, which took about two hours

158 to complete.

### 159 **Gas concentration measurement**

160 The sample air stream from each of the eight reactors was measured sequentially for 10 min  
161 before switching to another reactor. The background air sample flowing directly from the air  
162 supply manifold was sampled for 30 min. It required 110 min (1 h and 50 min) to scan through  
163 the background air and the eight reactors. Slightly more than six measurements were obtained  
164 daily for each of these eight reactor during typical measurement days, excluding days for weekly  
165 manure addition and system maintenance.

166 At the beginning of the study, NH<sub>3</sub> concentration in sample air was measured with an  
167 ammonia analyzer (Model 17C, Thermal Environmental Instruments, Inc., Franklin, MA, USA),  
168 which first converted NH<sub>3</sub> into nitric oxide (NO) then measured the NO concentration with a  
169 chemiluminescence detector. The analyzer had a lower detectable limit of 1 part per billion (ppb).  
170 Its 24-hour span drift was 1% of full scale. The NH<sub>3</sub> analyzer was set at 0-200 ppm measurement  
171 range. However, the analyzer was pegged during the first day of measurement with unexpectedly  
172 high NH<sub>3</sub> concentrations (>200 ppm) in the reactor exhaust air. A Chillgard IR Refrigerant Leak  
173 Detection System (Mine Safety Appliances Company, Pittsburgh, PA, USA) was therefore  
174 installed on day 2. This instrument was based on the photoacoustic infrared sensing technology.  
175 Its display resolution was 1 ppm with a measurement range of 0 to 1000 ppm and response time  
176 was 90% of a step-change in 70 seconds. Its stability was ± 1 ppm in 0–100 ppm measurement  
177 and ± 10% of reading in 100–1000 ppm measurement.

178 A photoacoustic infrared carbon dioxide (CO<sub>2</sub>) analyzer (Mine Safety Appliances,  
179 Pittsburgh, PA, USA) was installed and used throughout the study to measure CO<sub>2</sub> concentrations.  
180 Its normal measurement range was 0–5000 ppm and was extended to 0–10,000 ppm for this study  
181 by adjusting its sensitivity as suggested by the instrument manufacturer. The precision of the



182 analyzer was  $\pm 100$  ppm. Carbon dioxide concentration was initially used as an indicator to  
183 compare with the  $\text{NH}_3$  concentration for research quality control. For example, if both  $\text{CO}_2$  and  
184  $\text{NH}_3$  were not detected, it could be an indication of sampling system failure.

185 A hydrogen sulfide analyzer (Model 45C, TEI, Franklin, MA, USA) was also installed for  
186  $\text{H}_2\text{S}$  measurement (Figure 2 錯誤! 找不到參照來源。). The analyzer detection limit was 2.0 ppb  
187  $\text{H}_2\text{S}$ . Its precision was 1% of the reading or 1 ppb.

188 All the gas analyzers were calibrated or zero/span checked prior to and after the study, and  
189 at least weekly during the study using certified zero air, and  $\text{NH}_3$ ,  $\text{CO}_2$ , and  $\text{H}_2\text{S}$  calibration gases.

#### 190 **Airflow rate, temperature, relative humidity, and pressure measurement**

191 A mass-flow meter with  $0\text{--}10\text{ L min}^{-1}$  measurement range (Model 50S-10, McMillan,  
192 Georgetown, TX, USA) was used to measure the airflow rate from each reactor when the gas  
193 concentrations of that reactor were being measured.

194 Air temperature in the reactor room was monitored in four locations with type T  
195 thermocouples. An electronic relative humidity and temperature probe (Humitter 50 YC, Vaisala,  
196 Woburn, MA, USA) was used inside the air supply manifold ( $M_a$ ) to monitor relative humidity  
197 and temperature.

198 Six pressure sensors were installed to monitor the test system. The first sensor measured the  
199 pressure inside manifold  $M_a$ . The second monitored the pressure in the air sampling manifold  
200 ( $M_s$ ). The third monitored the pressure at the exhaust air of the distribution manifold ( $M_d$ ) to  
201 ensure sufficient air supply during analyzer calibration (Figure 2). The three other sensors  
202 monitored static pressures of the exhaust hood, the reactor room, and the instrumentation room,  
203 respectively.

#### 204 **Data acquisition and control**

205 The data acquisition and control (DAC) system consisted of a personal computer and

206 FieldPoint DAC hardware (National Instruments, Austin, TX, USA). The DAC program for this  
207 study was written in LabVIEW (National Instruments). Measurement data were sampled every  
208 second and averaged and recorded every minute. The DAC program controlled the solenoids for  
209 automatic air sampling (Ni and Heber, 2010).

## 210 **Odor evaluation**

211 To evaluate the additive effectiveness on odor reduction and assess the performance claim  
212 that the sponsor received from the additive vendor, the odor intensities of the reactor exhaust air  
213 were evaluated by four trained odor panelists before and after the day-14 additive application.  
214 Each panelist directly sniffed the reactor exhaust air, and recorded an odor intensity based on  
215 comparison with a reference scale of n-butanol solutions (ASTM Committee E-18, 1992). The  
216 concentrations of n-butanol in water of the 5-point scale reference were 250, 750, 2250, 6750,  
217 and 20,250 ppm for levels 1 to 5, respectively. The panelists were allowed to score at 0.5 level  
218 increment. The odor intensities of the control and treated reactors were sniffed before and after  
219 application of the additive. This comparison of odor intensities was also used to evaluate the  
220 longer term (7 d) effectiveness of two previous additive applications on days 0 and 7.

## 221 **Open-headspace tests**

222 Open-headspace tests were conducted to simulate commercial demonstrations of the  
223 additive product and to corroborate the results of the reactor study. Manure was tested twice  
224 using a 38-cm diameter and 8-cm deep pan on days 21 and 28. Layer hen manure taken from the  
225 frozen supply of source manure and thoroughly mixed was placed in the pan. An inverted funnel  
226 (10-cm diameter opening) was held 1.5-cm above the surface of the manure in the pan to collect a  
227 continuous air sample. The funnel was connected to the 17C NH<sub>3</sub> analyzer and the CO<sub>2</sub> analyzer  
228 for continuous measurement. The vacuum pumps of the analyzers drew sample air continuously  
229 from the funnel at about 2 L min<sup>-1</sup>

230 On the day 21 test, 17 mL of additive solution was sprayed directly onto the surface of 1.1  
231 kg layer hen manure in the pan, which was placed on the floor of the laboratory. Gas  
232 concentrations were compared before and after the spray. The test was repeated on the same day  
233 with 2.3 kg of manure. Additive solution (15 mL) was sprayed once onto the manure to compare  
234 their effects. The measurement continued overnight for a total of 13 h.

235 On the day 28 test, 3.1 kg of layer hen manure was used in the pan, which was placed  
236 inside a 40 cm (H) x 43 cm (W) x 66 cm (L) plastic box to reduce the effect of room air  
237 circulation on the gas release from manure. Gas measurements were made continuously for 25 h;  
238 and 15 mL of additive solution was sprayed onto the manure 3 hours after the measurement  
239 started.

#### 240 **Data processing and analysis**

241 In data processing, only the last 3 min of gas concentration data during the 10 min or 30  
242 min measurement were used. The first 7 or 27 min data were ignored because these were the time  
243 that the measurement system needed to equilibrate after switching from one sampling location to  
244 another. Gas release rate was calculated by multiplying the airflow rate by the gas concentration  
245 difference between the reactor exhaust air and the reactor inlet air, after converting from  
246 volumetric concentration (ppb or ppm) to mass concentration ( $\mu\text{g m}^{-3}$ ). Gas release flux was  
247 calculated by dividing the release rate by  $0.114 \text{ m}^2$  of the reactor top surface (38-cm diameter),  
248 not the actual poultry manure surface areas exposed to the air. The actual manure surface was  
249 rough and irregular and was technically impossible to determine in this study.

250 Several means were defined in this study depending on the coverage of time duration (4 h,  
251 1 day, and 38 days) and number of reactors (1 reactor and 4-reactor group). For example, a  
252 “group daily mean” was calculated for a 4-reactor group with 1 day of data; and an “average  
253 reactor mean” was calculated for a single reactor from 38 days of data (Table 2).

254 Table 2: Means defined and used in the data analysis.

Time coverage	Reactor coverage		
	Individual reactor	4-reactor group	8 reactors
4-h sample	Reactor sample	Group sample mean	8-reactor sample mean
1 day	Reactor daily mean	Group daily mean	8-reactor daily mean
38 days	Average reactor mean	Average group mean	Average 8-reactor mean

255

256 Statistical t-Test (two-tailed unequal variances) was performed to compare the analysis  
 257 results of manure samples taken from the treated and control reactors. It was also used to  
 258 compare the manure odor intensities and the group daily means gas release rates between the  
 259 treated and control groups. Single factor ANOVA was used to compare the differences between  
 260 the eight reactors using reactor daily means.

## 261 RESULTS AND DISCUSSION

### 262 Manure characteristics

263 Results of manure analysis showed that the pH of three types of manure samples (i.e.,  
 264 source manure from hen House 1 sampled on day 0, reactor top manure from House 2 that was  
 265 added on day 28 and sampled on day 38, and reactor mixed manure sampled on day 38) was very  
 266 close and ranged from 8.69 to 8.79 (Table 3). Manure from House 2 had apparently higher  
 267 concentrations of moisture, total N, and ammonium than that from House 1.

268 However, no statistical differences were observed between the control reactors and the  
 269 treated reactors for all the analyzed variables (pH, moisture, total N, and ammonium) in reactor  
 270 top manure and mixed manure sampled on day 38 ( $P > 0.30$ ) (Table 3). This demonstrated that no  
 271 effects of the Eco-Cure application on manure characteristics were observed.

272

273 Table 3: Mean  $\pm$  standard deviation (STD) of manure analysis results.

Manure sample and T-Test	Sample, n	Mean $\pm$ STD			
		pH	Moisture, %	Total N, ppm	NH <sub>4</sub> <sup>+</sup> , ppm
<b>Source manure from House 1</b>					
Control and treated reactors on day 0	3	8.79 $\pm$	33.85 $\pm$ 2.29	18,056 $\pm$ 912	5296 $\pm$

			0.06		652
<b>Top manure from House 2</b>					
Control reactors on day 38	4	8.73 ± 0.08	43.02 ± 5.28	27,541 ± 3078	8860 ± 740
Treated reactors on day 38 *	3	8.78 ± 0.08	40.53 ± 2.69	25,247 ± 4198	8126 ± 653
Control vs. treated (P value)		0.51	0.52	0.55	0.30
<b>Mixed manure from Houses 1 and 2</b>					
Control reactors on day 38	12	8.69 ± 0.07	37.03 ± 2.64	19,481 ± 1531	6497 ± 607
Treated reactors on day 38	12	8.71 ± 0.08	37.5 ± 3.31	19,162 ± 1882	6848 ± 943
Control vs. treated (P value)		0.66	0.72	0.67	0.31

274 Note: \* Day 38 R-g top manure was not available due to a sample error.

## 275 **Reactor gas concentration and release**

### 276 *Data overview*

277 Between 214 and 223 group sample means of NH<sub>3</sub> and CO<sub>2</sub> concentrations and calculated  
 278 releases were obtained for the control and treated groups (Table 4). Hydrogen sulfide  
 279 concentrations were below the detection limit of the gas analyzer. This agreed with other field  
 280 studies, which demonstrated that H<sub>2</sub>S concentrations and emissions at high-rise layer hen houses  
 281 were very low compared with commercial pig buildings (Ni et al., 2012; 2017). Therefore, H<sub>2</sub>S  
 282 concentrations and releases between control and treated reactors could not be compared in this  
 283 study.

284 Table 4: Averaged group mean (AGM) ± standard deviation (STD) of ammonia and carbon  
 285 dioxide concentrations and releases from the control and treated reactors.

Parameters	Control group (R-a to R-d)		Treated group (R-e to R-h)	
	AGM ± STD	GSM, n	AGM ± STD	GSM, n
NH <sub>3</sub> concentration	196 ± 161 ppm	222	229 ± 143 ppm	223
NH <sub>3</sub> release rate	17.5 ± 14.3 µg s <sup>-1</sup>	222	20.1 ± 12.6 µg s <sup>-1</sup>	223
NH <sub>3</sub> release flux	153.7 ± 125.3 µg s <sup>-1</sup> m <sup>-2</sup>	222	176.6 ± 110.4 µg s <sup>-1</sup> m <sup>-2</sup>	223
CO <sub>2</sub> concentration	5288 ± 740 ppm	214	5559 ± 1103 ppm	215
CO <sub>2</sub> release rate	1091 ± 149 µg s <sup>-1</sup>	214	1143 ± 217 µg s <sup>-1</sup>	215
CO <sub>2</sub> release flux	9566 ± 1310 µg s <sup>-1</sup> m <sup>-2</sup>	214	10024 ± 1904 µg s <sup>-1</sup> m <sup>-2</sup>	215

286 Note: Group sample means (GSM) were used to calculate 38 group daily means, which were then  
 287 used to calculate averaged group means.

### 288 *Effect of additive on ammonia and carbon dioxide concentration and release*

289 Compared with the control group, the treated group NH<sub>3</sub> had about 16.8% higher average

290 concentration and 14.9% higher average release rate (Table 4). However, statistically there were  
291 no significant differences for both NH<sub>3</sub> concentrations (P = 0.35) and releases (P = 0.41). The  
292 additive was therefore not effective in reducing NH<sub>3</sub> release from layer hen manure in this study.

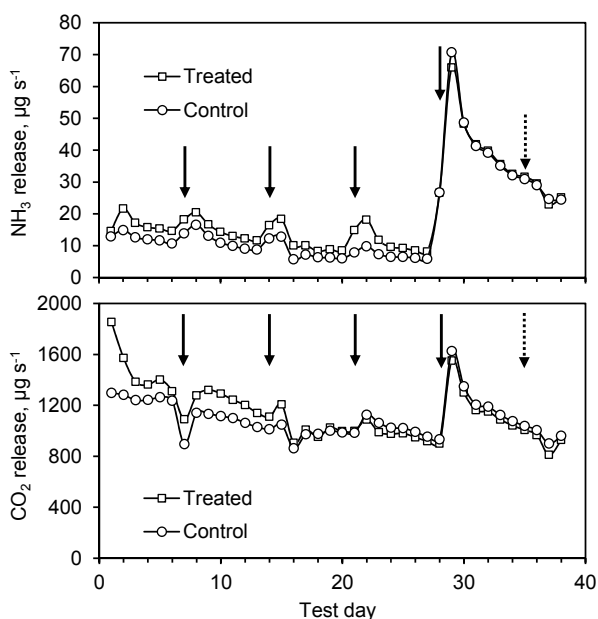
293 The treated group CO<sub>2</sub> had about 5.1% higher average concentration and 4.8% higher  
294 average release rate compared with the control group (Table 4). However, these differences were  
295 not statistically significant (P > 0.21 for CO<sub>2</sub> concentration and P > 0.23 for CO<sub>2</sub> release rate).  
296 Therefore, no effects of the additive on CO<sub>2</sub> from manure were observed.

### 297 *Temporal variations of ammonia and carbon dioxide releases*

298 The daily mean NH<sub>3</sub> releases increased substantially after each weekly manure addition,  
299 despite the immediate additive application, followed by a gradual decrease until the subsequent  
300 addition (Figure 3, top). Nevertheless, the peaks that occurred after the first three additions of  
301 stored manure on days 7, 14 and 21 were much lower than those after the last addition of one-day  
302 old fresh manure on day 28, which produced peaks that were more than 3.5 times as high as the  
303 previous additions. The day 28 manure from House 2 had higher moisture, total N, and  
304 ammonium concentrations compared with the day 0 source manure from House 1 (Table 3),  
305 demonstrating that different poultry manure could have very different NH<sub>3</sub> release potentials.  
306 Consequently, the NH<sub>3</sub> release rate after the day-28 manure addition and additive application was  
307 1330% as high as before the addition, demonstrating that fresher manure with higher moisture  
308 concentrations had substantially higher NH<sub>3</sub> release potentials. However, no reductions in NH<sub>3</sub>  
309 concentrations or releases were observed immediately after each additive spray application in the  
310 reactors. Therefore, this additive did not exhibit an immediate effect on NH<sub>3</sub> release from layer  
311 hen manure as claimed by the manufacturer.

312 Similar behaviors of CO<sub>2</sub> release from the manure were also observed (Figure 3, bottom).  
313 The CO<sub>2</sub> releases increased after the manure additions and additive applications, and decreased

314 gradually thereafter, showing the same fluctuation patterns as NH<sub>3</sub>. Although the increase in CO<sub>2</sub>  
 315 release after the fourth weekly addition with manure of higher moisture concentration was much  
 316 more intense relative to the three previous additions, it was not as high as that of the NH<sub>3</sub> release.  
 317 However, additive application did not noticeably alter the CO<sub>2</sub> release patterns for the treated  
 318 reactors compared with the control reactors.



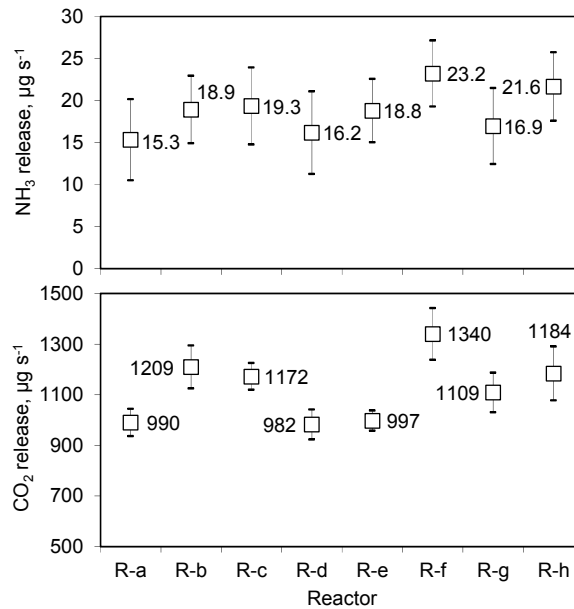
319  
 320 Figure 3. Group daily mean release rates of ammonia (top) and carbon dioxide (bottom) from the  
 321 treated and control groups. Solid arrows: manure additions and additive applications. Dotted  
 322 arrows: additive application only.

323 ***Ammonia and carbon dioxide release among individual reactors***

324 The average reactor mean NH<sub>3</sub> releases from the manure in the eight individual reactors  
 325 ranged from 15.3 µg s<sup>-1</sup> (R-a) to 23.2 µg s<sup>-1</sup> (R-f) (Figure 4, top). According to the ANOVA  
 326 analysis results, no significant differences were found for NH<sub>3</sub> releases between the eight reactors  
 327 (P = 0.20). The variations in NH<sub>3</sub> releases cannot be obviously related to the effect of the additive,  
 328 but most possibly to the variations of the manure sources, which were randomly assigned to each  
 329 reactor (Table 3). Similar variations in NH<sub>3</sub> concentrations among different reactors was  
 330 observed in previous studies using swine manure, dairy manure, beef manure, and municipal

331 sludge (Tengman et al., 2001; Dunn, 2004; Dai et al., 2015).

332 The average reactor mean CO<sub>2</sub> releases from the manure in individual reactors ranged from  
333 982 μg s<sup>-1</sup> (R-d) to 1340 μg s<sup>-1</sup> (R-f) (Figure 4, bottom). Compared with NH<sub>3</sub>, the variations in  
334 CO<sub>2</sub> releases from manure among different reactors were more profound. The ANOVA analysis  
335 results showed significant differences for CO<sub>2</sub> releases between the eight individual reactors (P <  
336 0.001). However, these CO<sub>2</sub> release variations were randomly distributed and could not be related  
337 to the effects of additive applications.



338  
339 Figure 4. Average reactor mean ± 95% confidence interval of ammonia (top) and carbon dioxide  
340 (bottom) releases in the control reactors (R-a to R-d) and treated reactors (R-e to R-h).

### 341 Odor intensity

342 The group mean odor intensities of the reactor exhaust air were 3.4 ± 0.3 and 3.5 ± 0.3 for  
343 the control and treated groups, respectively, before the day-14 additive spray using the 5-point n-  
344 butanol reference scale. No effect on odor intensity reduction by the additive applications on days  
345 0 and 7 was demonstrated (P > 0.71). The odor intensities of the treated reactors were 3.5 ± 0.3  
346 and 3.4 ± 0.3 before and after the additive spray, respectively. No statistically significant  
347 difference was observed (P > 0.77) to reject the null hypothesis that the additive did not reduce

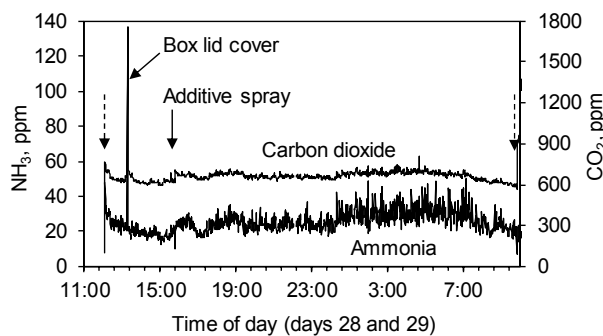


348 odor intensities in the air from layer hen manure based on the odor sniffing test.

### 349 **Open-headspace gas release**

350 During the day 21 open-headspace test, concentrations of  $\text{NH}_3$  and  $\text{CO}_2$  above the manure  
351 surface gradually decreased after the monitoring began. However,  $\text{NH}_3$  concentrations started to  
352 increase 90 min after the additive spray and stayed at about 55 ppm for 3 h before it decreased  
353 again and remained at about 10 ppm overnight. It was noticed that air movement in the lab due to  
354 research activities may have contributed to the concentration variations. Nevertheless, an  
355 immediate elimination of  $\text{NH}_3$  after additive spray as claimed for the additive product was not  
356 observed.

357 The day 28–29 open-headspace test confirmed the results of the day 21 test that the additive  
358 spray at 15:45 did not reduce  $\text{NH}_3$  release (Figure 5). The gas concentrations in this test were  
359 more stable than the day 21 test and  $\text{NH}_3$  remained at about 30 ppm throughout the test due to the  
360 use of the plastic box, from which the released gases flowed out of the box by convection.  
361 Concentrations of both  $\text{NH}_3$  and  $\text{CO}_2$  suddenly increased to 137 ppm and 1450 ppm, respectively,  
362 when the top of the box was briefly covered for 1 min, manifesting high release rates of the gases  
363 from manure (Figure 5).



364

365 Figure 5. Results of open-headspace test on days 28 and 29. The left and right dashed arrows  
366 indicate the start and end of the test, respectively.

## 367 **Comparison between laboratory simulation and field condition**

### 368 ***Ventilation***

369 The ventilation flux on manure surface in this study was  $64 \text{ L min}^{-1} \text{ m}^{-2}$ . The measured  
370 minimum and mean ventilation in commercial layer hen houses were  $295$  and  $2420 \text{ L min}^{-1} \text{ m}^{-2}$ ,  
371 respectively (Heber et al., 2005). The volumetric air exchange rate in the reactor headspace  
372 varied from  $6.7$  to  $10.4$  air changes per hour (ach) compared with the minimum and maximum  
373 layer hen house ventilation rate of  $4.7$  and  $53$  ach, respectively. Therefore, whereas the  
374 volumetric airflow rate in the study simulated winter ventilation rates in the layer hen house,  
375 surface based ventilation rate was generally lower than in the layer hen houses. The surface to  
376 volume ratio in the reactor ranged from  $1.8$  to  $2.8 \text{ m}^2 \text{ per m}^3$  compared with about  $0.20 \text{ m}^2 \text{ per m}^3$   
377 in the layer hen house. Thus surface to volume ratio was about 8–13 times higher in the reactors  
378 than in the houses. However, the higher concentrations of  $\text{NH}_3$  in the reactors were mainly due to  
379 the relatively lower surface-specific ventilation compared with the layer hen house situations, and  
380 most of the  $\text{NH}_3$  release is expected to be from fresh manure on the surface of the manure  
381 windrows.

### 382 ***Ammonia concentration and release***

383 The AGM  $\text{NH}_3$  concentrations in the reactor exhaust air were much higher than that  
384 measured in the exhaust air of the commercial layer hen farm, from which the reactor manure  
385 was obtained. The  $\text{NH}_3$  concentrations measured in two of the 250,000-hen high-rise layer hen  
386 houses at the farm averaged  $34$  ppm in a 380-d long continuous measurement campaign with the  
387 maximum daily average of  $108$  ppm (Heber et al., 2005). However, the average reactor mean  
388  $\text{NH}_3$  concentrations were close to the maximum daily mean  $\text{NH}_3$  concentrations, which were  $176$   
389 and  $182$  ppm, in two other reported high-rise layer hen houses (Ni et al., 2012), showing at least  
390 some similarities between the laboratory and field conditions.

391 The average 8-reactor mean of  $\text{NH}_3$  release flux was  $164 \mu\text{g s}^{-1} \text{m}^{-2}$ , which was only about  
392 26% of the  $622 \mu\text{g s}^{-1} \text{m}^{-2}$  of manure pit floor area in the layer hen houses (Heber et al., 2005).  
393 Field data, however, included releases from the cage area inside the layer hen houses. The  
394 inverted V-shaped manure windrows in high-rise layer hen houses also had much large manure  
395 surface areas than the pit floor area. Additionally, the layer hen houses had fresher manure that  
396 dropped frequently onto the top of manure piles and ventilation rates that varied diurnally and  
397 seasonally compared with the laboratory setup. Therefore, higher  $\text{NH}_3$  release flux per pit floor  
398 area in the layer hen houses could be expected.

## 399 **CONCLUSIONS**

400 The following conclusions were obtained in this study:

401 1. Applications of Eco-Cure in four laboratory reactors did not alter manure pH, moisture,  
402 total nitrogen, and ammonium in the treated manure compared with manure in four control  
403 reactors.

404 2. Reductions in  $\text{NH}_3$  releases from layer hen manure after the additive application were not  
405 observed.

406 3. No effects of the additive on reducing odor intensity of layer hen manure were observed  
407 by the odor panel.

408 4. Spraying the additive solution onto manure surface in the open-headspace did not  
409 demonstrate immediate reduction on  $\text{NH}_3$  and  $\text{CO}_2$  concentrations near the manure surface.

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## Figure Captions

492

493 Figure 1: The reactor. The “top cap” was removed during manure addition, additive spray,  
494 and reactor emptying. The position of the air supply pipe was adjustable (indicated with the  
495 vertical double-ended arrow).

496 Figure 2: Laboratory setup airflow diagram. Three of the gas analyzers had internal filters  
497 and/or pumps.

498 Figure 3. Group daily mean release rates of ammonia (top) and carbon dioxide (bottom)  
499 from the treated and control groups. Solid arrows: manure additions and additive applications.  
500 Dotted arrows: additive application only.

501 Figure 4. Average reactor mean  $\pm$  95% confidence interval of ammonia release (top) and  
502 carbon dioxide release (bottom) from the control reactors (R-a to R-d) and treated reactors (R-e to  
503 R-h).

504 Figure 5. Results of open-headspace test on days 28 and 29. The left and right dashed  
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